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Interaction between mycorrhization with *Glomus intraradices* and phosphorus in nursery olive plants

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ABSTRACT

Mycorrhization of nursery plants with arbuscular mycorrhizal fungi (AMF) has become a usual practice in olive nurseries. Mycorrhizal associations increase plant tolerance to biotic and abiotic stresses after transplanting by improving root system structure and development. Nutrient fertilization is also desirable because roots are confined in a limited amount of soil in the nursery containers. Since the olive tree is a species with low phosphorus requirements, and AMF can affect root system development and nutrient uptake, it is of interest to study the possible interaction between both factors. Three different experiments with potted plants growing in a shade house, and one experiment under field conditions, were preformed using young olive plants to explore these questions. Inoculation with *Glomus intraradices* did not affect the phosphorus level in the plants but reduced shoot growth in plants growing in phosphorus-rich soil. When the substrate was a soil poor in phosphorus, shoot growth of AMF-inoculated plants was similar to the control but root development was greater. Mycorrhization also increased flower number and quality in 'Arbequina'. Shoot growth also was reduced when a sterilized substrate was used, suggesting that the use of natural soil is preferred. Since no mycorrhization effect was observed when inoculation was performed at the moment of transplanting to the field, inoculation during the nursery-growing period is recommended to improve plant quality for orchard establishment.

1. Introduction

Spain is the main world olive oil producer, accounting for more than a third of worldwide production with over 1402 thousand metric tons (IOC, 2017). In Andalusia (Southern Spain), olive orchards are widely extended (1.6 million ha), representing 60% of the national olive growing area (MAPAMA, 2017). In this region olive growing includes diverse olive farming systems, ranging from extensive monoculture to marginal cultivation or modern intensive and high-density orchards. Due to improved farming methods and increased awareness of the health benefits of olive oil many older, more traditional orchards are being renewed, as well as the area where the crop is grown expanding to many new countries. New plantations, some of them with high plant densities (1500–2000 trees ha^{-1}), require a large number of plants which are supplied annually by commercial olive nurseries.

New olive orchards are usually established with mist-propagated plants derived from softwood cuttings. The rooted cuttings are placed in plastic bags containing substrate for a period of further growth. These plants are usually fertilized during the nursery growing period with a compound fertilizer containing several mineral elements. Fertilization of the containerized plants is required because the roots are confined in a limited amount of soil and rapid vegetative growth depletes the available minerals. Since the olive tree is a particularly mycotrophic plant (Roldan-Fajardo and Barea, 1986), in recent years many new olive orchards have been established with plants to which commercial arbuscular mycorrhizal fungi (AMF) have been incorporated in the potting media during the nursery period to promote root system development and facilitate establishment of the new orchards (Azcón-Aguilar and Barea, 1997; Calvente et al., 2004; Binet et al., 2007; Dag et al., 2009; Meddad-Hamza et al., 2010). It is well known that mycorrhizal associations increase plant tolerance to drought stress (Wu et al., 2013) and salinity (Porras-Soriano et al., 2009), protect from nematode attacks (Castillo et al., 2006), and tend to increment nutrient uptake (Dag et al., 2009; Chatzistathis et al., 2013).

Phosphorus (P) is an essential element for plant growth and, consequently, is a limiting factor under deficiency conditions. The olive tree, however, is a species with low phosphorus requirements, as occurs in other woody plants (Rennenberg and Herschbach, 2013), including

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Table 1
Analytical characteristics of the soils used. The high P soil was from the experimental farm of de Rabanales Campus, University of Córdoba, Spain, and the low P soil from a field at Santa Cruz (Córdoba province).[1]

	HH[2] (%)	$CaCO_3$	EC[3] (1.5) (dS/m)	P Olsen (mg/kg)	Clay (%)	Lime (%)	Sand (%)
Low P soil	7.4 ± 0.01[4]	43.5 ± 0.4	0.14 ± 0.00	4.2 ± 0.2	40.0 ± 0.4	27.5 ± 0.7	32.5 ± 1.0
High P soil	7.1 ± 0.08	1.5 ± 0.6	0.13 ± 0.01	26.1 ± 2.7	36.33 ± 0.9	18.7 ± 0.3	45.0 ± 1.0

[1] The high P soil was used in Experiments 2 and 4. Both soils were used in Experiment 3.
[2] Hygroscopic humidity.
[3] Electrical conductivity.
[4] Values expressed as mean ± SE.

Table 2
Phosphorus concentration in leaves, stems and roots and total plant content according to mycorrhization and P applied in Experiment 1.

	P concentration (% dw)					P plant content (mg)
	New leaves	Old leaves	New stems	Old stems	Roots	
	Mycorrhization[1]					
−AMF	0.13 a	0.13 a	0.14 a	0.13 a	0.31 a	33.96 a
+AMF	0.13 a	0.12 a	0.12 a	0.12 a	0.31 a	34.23 a
	P applied (ppm)					
0	0.11	0.10	0.11	0.10	0.12	21.29
100	0.13	0.12	0.13	0.13	0.33	38.32
200	0.14	0.14	0.14	0.14	0.48	42.68
Significance[2]	L**	L**	NS	L***	L***,Q*	L***,Q*
CV (%)[3]	6.2	6.9	11.1	10.4	14.2	4.6

[1] Letters indicate mean separation within each column at P ≤ 0.05, by F test.
[2] NS, *, **, *** Non-significant or significant at P ≤ 0.05, ≤0.01, or ≤0.001, respectively; L = linear, Q = quadratic.
[3] Coefficient of variation.

Table 3
Total increase in shoot growth four and twelve months after the initiation of treatments according to mycorrhization (± AMF) and P applied in Experiment 1.

	Shoot growth (cm)	
	Four months	Twelve months
	Mycorrhization[1]	
−AMF	65.13 a	98.45 a
+AMF	56.49 b	83.60 b
	P applied (ppm)	
0	56.83	83.13
100	62.26	92.14
200	63.35	97.76
Significance[2]	NS	NS
CV (%)[3]	5.7	2.9

[1] Letters indicate mean separation within each column at P ≤ 0.05, by F test.
[2] NS = non-significant.
[3] Coefficient of variation.

young olive plants growing in pots (Jiménez-Moreno and Fernández-Escobar, 2016). Excess of phosphorus might also have adverse effects. Jiménez-Moreno and Fernández-Escobar (2016) described P toxicity levels and symptoms in young olive plants. Furthermore, some works have reported a possible reduction of AMF colonization caused by phosphorus application in vineyards (Schreiner and Linderman, 2005; Schreiner, 2010). If this negative interaction were to occur due to P fertilization of potted nursery olive plants, the benefits from mycorrhization of those plants could be lost.

The objectives of the present work were to study the effect of mycorrhization of nursery olive plants and its interaction with phosphorus nutrition, and to assess the results of inoculation with AMF under different levels of phosphorus both in the nursery and in the field.

Table 4
Phosphorus concentration in leaves, stems and roots and total plant content according to mycorrhization (± AMF) and P applied in Experiment 2.

	P concentration (% dw)					P plant content (mg)
	New leaves	Old leaves	New stems	Old stems	Roots	
	Mycorrhization[1]					
−AMF	0.14 a	0.15 a	0.13 a	0.08 a	0.18 a	29.45 a
+AMF	0.13 a	0.13 a	0.12 a	0.08 a	0.17 a	28.99 a
	P applied (ppm)					
0	0.12	0.12	0.12	0.08	0.11	25.40
200	0.15	0.16	0.13	0.09	0.24	33.04
Significance[2]	L**	L**	NS	NS	L***	L*
CV (%)[3]	6.2	6.9	11.1	10.4	14.2	4.6

[1] Letters indicate mean separation within each column at P ≤ 0.05, by F test.
[2] NS, *, **, *** Non-significant or significant at P ≤ 0.05, ≤0.01, or ≤0.001, respectively; L = linear.
[3] Coefficient of variation.

Table 5
Total increase in shoot growth at the end of the Experiment 2.

	Shoot growth (cm)
	Mycorrhization[1]
−AMF	27.70 a
+ AMF	32.14 a
	P applied (ppm)
0	34.14
200	25.66
Significance[2]	L**
CV(%)[3]	4.9

[1] Letters indicate mean separation within each column at P ≤ 0.05, by F test.
[2] **Significant at P ≤ 0.01; L = linear.
[3] Coefficient of variation.

2. Materials and methods

2.1. Experimental design

Four different experiments were carried out to study the effect of mycorrhization on young olive plants. A preliminary first experiment was conducted with mist-rooted 'Picual' olive plants growing in 1.1 L pots in a shade house. A sterilized mixture of washed river sand and peat (2:1 by volume) was used as potting medium, to which the inoculum was added for the mycorrhization treatments. Plants were watered one-two times a week, depending on their requirements, with 100 mL water per pot, and monthly with a nutrient solution without P to prevent deficiencies of other nutrients. This solution was composed of 2.5 mM $Ca(NO_3)_2$, 2.5 mM KNO_3, 1.0 mM $MgSO_4$, 12.5 mM H_3BO_3, 1.0 mM $ZnSO_4$, 0.2 mM $(NH_4)_6Mo_7O_{24}$, 10 mMFe-ethylenediamine-di-o-hydroxy-phenylaceticacid, and 0.5 mM KCl. The experiment was arranged in a factorial design with two factors and ten replications. One factor was plant mycorrhization (−AMF or +AMF), and the other was